SHORT PAPERS

N-NITROSODIMETHYLAMINE IN HUMAN BLOOD

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Abstract—Whole blood from 38 healthy persons (19 males and 19 females) was analysed for volatile nitrosamines. *N*-Nitrosodimethylamine was detected in blood samples from 97% of the individuals tested at a mean value (±1 SD) of 0·6 ± 0·4 ng/ml.

Introduction

Volatile nitrosamines have been reported in the urine (Brooks, Cherry, Thacker & Alley, 1972; Hicks, Gough & Walters, 1978; Radomski, Greenwald, Hearn, Block & Woods, 1977) and faeces (Wang, Kakizoe, Dion, Furrer, Varghese & Bruce, 1978) of healthy individuals, in gastric contents from patients with gastro-intestinal disorders (Lakritz, Wasserman, Gates & Spinelli, 1978), and in the blood from one individual (Fine, Ross, Rounbehler, Silvergleid & Song, 1977). These findings suggest that *in vivo* nitrosation may occur in man. Because of the potent carcinogenic properties of nitrosamines, as shown by animal studies (Magee & Barnes, 1967), it would be important to demonstrate the presence of dialkylnitrosamines in the blood. This paper reports on the evidence for volatile nitrosamines obtained from examination of blood from normal men and women and on the detection of *N*-nitrosodimethylamine (NDMA) in 97% of the individuals tested.

Experimental†

Blood sampling. Blood samples from 38 normal subjects (19 men and 19 women, aged 21–60 yr; mean age 38 yr) were analysed for volatile nitrosamines. A syringe was used to collect all blood samples, which were immediately transferred to glass beakers and made basic. Commercially available evacuated blood-collection tubes were not used to collect the blood samples because we found that some rubber stoppers used to seal these tubes contained volatile nitrosamines (Lakritz & Kimoto, 1980). Additional fasting blood samples from one individual were collected periodically over a 6-month period to ascertain any variation in nitrosamine content.

Extraction procedure. Prior to initiating the study, the extraction of volatile nitrosamines in whole blood by distillation from alkali was compared with the use of a column-chromatographic technique using Na_2SO_4 and Celite without the application of heat. Our results indicated that both procedures were quantitatively comparable and that neither induced the formation of artefacts.

Analysis. In preliminary studies, we found that NDMA and the internal standard (*N*-nitrosomethylethylamine) were equally distributed in the cellular elements of the blood and in the plasma or serum. Therefore, it was advantageous to analyse whole blood samples, permitting work on samples as small as 10 ml. The volatile nitrosamines were analysed by a modification of a procedure described by Telling, Bryce & Althorpe (1971), involving distillation of whole blood from an alkaline solution. The distillate was collected and extracted with methylene chloride and the extracts were concentrated by evaporation. Analyses were performed with a gas–liquid chromatograph (GC) interfaced with a Thermal Energy Analyzer (TEA) Model 502 (Waltham, MA), a selective nitrosamine detector, operated under conditions similar to those employed by Fine & Rounbehler (1975). The levels of NDMA were determined by comparison with known concentrations of NDMA found to be linear over a range 0·1–10·0 ng/ml; the minimum confidence level of detection was 0·1 ng/ml. Where apparent nitrosamines were identified on the basis of GC retention time and TEA detectability, further confirmation was obtained by ultraviolet photolysis (Doerr & Fiddler, 1977).

Several preliminary experiments were conducted to ensure the accurate determination of concentrations of detectable nitrosamines in blood. Neither changes

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†Note: Precaution should be exercised in the handling of nitrosamines since they are potential carcinogens.

Table 1. N-*Nitrosodimethylamine in whole blood from healthy subjects*

Subjects	No. tested	*N*-Nitrosodimethylamine (ng/ml)* Range	Mean ± 1 SD
Total	38	0–1·5	0·6 ± 0·4
Male	19	0·2–0·8	0·4 ± 0·2
Female	19	0–1·5	0·8 ± 0·4
Fasted	25	0–1·5	0·6 ± 0·4
Non-fasted	13	0·2–1·2	0·5 ± 0·3
Smokers	11	0–1·2	0·7 ± 0·3
Non-smokers	27	0·2–1·2	0·5 ± 0·3

*ng/ml ≅ ppb ≃ μg/kg.

in the concentration of nitrosamines nor formation of artefacts were observed when 3 N-KOH or heparin was added to freshly drawn blood and the blood was stored in a frozen state for up to 3 days. All reagents and sample blanks used in this study were checked and found to be free from TEA-responsive peaks. Degassed deionized distilled water was used throughout the analyses. The errors associated with the whole-blood samples sizes of 10, 20 and 100 ml were found to be 0·15, 0·10 and 0·10 ng NDMA/ml, respectively. Ten determinations performed on the same blood specimen by three different analysts showed the NDMA content to be 0·3 ± 0·1 ng/ml (mean ± 1 SD). An internal standard, *N*-nitrosomethylethylamine, was added to each blood sample to ensure the reliability of each assay. Recoveries of the internal standard added at 1·0 and 2·0 ng/ml were 94 ± 5%. For statistical purposes, the blood-nitrosamine data were grouped according to sex, to whether samples were taken under fasting or non-fasting conditions and to whether the donors were smokers or non-smokers, and were subjected to analysis of variance.

Results

When blood samples from 38 subjects were assayed for volatile nitrosamines, 37 were found to contain NDMA. The results are shown in Table 1. The whole-blood NDMA level in these assays was 0·6 ± 0·4 ng/ml. Blood from one of the non-fasting males also contained 1·6 ng *N*-nitrosodiethylamine/ml. A higher concentration of NDMA appeared to be present in the blood of females (0·8 ± 0·4 ng/ml) than of males (0·4 ± 0·2 ng/ml). However, when fasting females were compared to fasting males no significant difference was observed. Neither were significant differences in the blood NDMA levels noted when smokers were compared to non-smokers. Fasting blood drawn periodically from one subject over a 6-month period showed a mean value of 0·5 ± 0·2 ng/ml.

Discussion

Volatile nitrosamines have been shown to be potent carcinogens when tested in a number of laboratory animals. Although no case of human cancer has been attributed to these compounds, recent reports have indicated that they are present in the urine (Brooks *et al.* 1972; Hicks *et al.* 1978; Radomski *et al.* 1977), in faeces (Wang *et al.* 1978), and in gastric contents (Lakritz *et al.* 1978). It is important, therefore, to determine whether nitrosamines are present in blood, whether they are of exogenous or endogenous origin (or both) and whether they are carcinogenic to man.

The data in this report addresses the first of these questions, and indicates that over 95% of the healthy individuals sampled for this study had measurable levels of NDMA in their blood.

The method used has a sensitivity of 0·1 ng/ml with a 95% recovery, so that the mean level of 0·6 ng/ml can be regarded as a valid result. These findings are comparable to results by Fine *et al.* (1977), who reported concentrations of 0·35 ng NDMA/ml in blood from a single subject before a meal of spinach, bacon, tomato, bread and beer, and 0·77 ng/ml 35 min later.

Nitrosamines in the blood may originate from ingestion of food containing preformed nitrosamines, from inhalation or from *in vivo* formation. Magee & Faber (1962) postulated a metabolic sequence for NDMA, as with other dialkylnitrosamines, which are also pre-carcinogens, with direct carcinogenic activity occurring only after activation through a series of demethylation and oxidative steps. The liver is probably the main metabolic site for this activation, and the presence of NDMA in human blood raises the question of the nitrosamine load and the rate of metabolic activation. The relationship of the findings reported in this study to the pathogenesis of cancer in man requires further investigation.

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